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METHODS OF MEASURING GROWTH RATE IN ALFALFA

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The undersigned hereby certify that they have read and recommend to the Committee on Graduate Studies for acceptance a thesis on "Methods of measuring growth rate in alfalfa", submitted by Warren E. Smith, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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METHODS OF MEASURING GROWTH RATE IN ALFALFA

Warren E. Smith

Department of Plant Science

A THESIS

submitted to the University of Alberta

in partial fulfilment of the

requirements for the degree of

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METHODS OF MEASURING GROWTH RATE IN ALFALFA

W. E. Smith

INTRODUCTION

Many workers have reported that alfalfa, like several other cross-breeding plants, exhibits reduced vigor of growth when inbred but maintains its vigor regularly under conditions of cross-pollination. Usually loss of vigor in alfalfa following inbreeding has been studied by comparison of inbred and open-pollinated plants with respect to yield of fodder at the "haying" stage of growth and with respect to seed yield. Comparisons of this kind have been made from field-plot yields obtained at one stage of growth only, viz. the early flowering or "haying" stage. It would seem desirable that loss of vigor due to inbreeding in alfalfa should be studied by much more refined methods involving careful measurements of growth rate at various stages of the plant's development before reliable generalizations can confidently be made. One of the difficulties in this procedure is the choice of the best criterion of growth rate in alfalfa. It was with the object of determining this best criterion that this investigation was undertaken. The criteria were conceived to involve methods of measurement and stages of plant development at which growth was measured. Several procedures

were adopted for purposes of comparison with each other and particularly with that of weighing the growth at different developmental stages, which is probably the best criterion, but which is not practical because of the necessity of removing the aerial parts of the plant for each measurement reading, which disrupts the continuity of the growth of the plant.

It was decided to study growth measurements in terms of radicle elongation, stem number, stem length, node number in the stem, and weight of aerial growth after a specified interval of time.

LITERATURE REVIEW

In the study of growth it is desirable to give some quantitative expression to the amount of growth which is accomplished by a plant or a group of plants during a given period of time. The principal measures which have been employed for this purpose, as outlined by Meyer and Anderson (13) are: (1) increase in the length of stem, root, or other organs of the plant; (2) increase in the area of leaves; (3) increase in diameter of stem (or other organs); (4) increase in volume; (5) dry weight increment; and (6) fresh weight increment.

If increase in length is taken as the measure,

Livingston (11) and Maximov (12) suggest that the simplest equipment for this study is a millimeter rule. A horizontal microscope or an auxanometer--a self-registering apparatus--may be used for finer and more accurate measurements of growth. If the growth of certain organs is used instead of that of the whole plant, they (11, 12) describe a method of marking the growing organs by means of India ink applied with a fine brush. These marks are made at definite distances from one another, and the increase in distance is measured at certain time intervals. This makes it possible to obtain a precise measurement of the changes in length of each portion during the experiment, and to ascertain the dimensions of the entire growing zone, since only in this region does the distance between the marks increase.

A photographic method for measuring and recording morphological and physical characters has been described by Pack (14). With the camera data for innumerable exact measurements and characters which may be used for future reference can be easily and quickly recorded. The photographic method possesses the advantage of permitting measurements of any degree of precision without the growing organ being touched, all disturbances of its normal development thus being eliminated.

However, the photographic method requires the use of controlled light, which may be impossible or undesirable.

Staniland (17) describes an apparatus which will yield similar information without the necessity of using controlled light. By the use of this apparatus accurate scale drawings can be made of plants for the study of growth changes; the investigator can make his drawings at suitable intervals of time, thus securing an accurate and permanent record of the development of the plant. It consists simply of a peep-hole punched through a piece of tin, and a piece of plain glass mounted in a frame. The glass is placed between the object to be drawn and the peep-hole so that by looking through the peep-hole the image of the object can be easily traced on the glass. It is a simple apparatus and has many useful applications.

Ipatjev (9), in his study of accurate morphological methods for measuring plant growth, suggested two methods. To one, the method of comparison of organs corresponding to one another, he gave the name "comparative ontogenetic method". For example, in a tomato plant the fifth real leaves which have attained their maximum development, ceased growing, and are liable to decay, are compared.

His second method, which may conveniently be called the "diagram method", consisted of tracing diagrammatically the progress of the plant's development. Here, the order of appearance of organs on a plant in the course of its development, is recorded. The differences in the expression of a

given organ are shown on the same graph, with the dates of their development.

It is often desirable to study the growth of roots and above-ground portions of the plant simultaneously. Goto (5), in his study of Halo Blight of oats, was able to do this by the use of glass-fronted tin cases, 22 x 12x 1.75 inches in size. These were placed at an angle of 45 degrees in specially constructed frames. This allowed the roots to grow against the glass so that daily observations and measurements could be made of roots and above-ground parts.

Arndt (2) was able to study all parts of the cotton seedlings which were grown in the dark in agar medium in test tubes, and was able to make daily observations on all parts of the seedling.

The growing of excised roots in a culture medium has proved to be a good method for comparing growth rates of different plants. Whaley and Long (23) studied the behavior of excised roots of heterotic hybrids and their inbred parents in culture media. They found that, in all cultural media the excised roots of hybrids of corn grew better than inbreds.

The measure of total weight is probably the best criterion of growth. Tysdal et al (20), in their review of alfalfa breeding, concluded that forage yield under field conditions is possibly the best criterion of the productivity of alfalfa.

Paddick (15) found that he was able to explain the dynamics of hybrid vigor in corn through measurement of linear growth of seedling radicle and immature leaves at comparable positions on plants of the same age. He measured the extent as well as speed of development by the number of leaves produced at a given time. He concluded that whatever hybrid vigor was found to be, it was safe to assume that the answer would be closely connected with the development of the plant.

Many workers have studied the relationship of size of seed to the subsequent growth of the plant. The work of Sprague (16), Kempton and McLane (10), Evans and Johnson (3) indicates that large seed size has little if any permanent significance. It seems that the measurement of seed size alone would not be a suitable criterion of growth.

Veatch (2) investigated several characters of soybeans with regard to hybrid vigor. He found that hybrids, on the average, exceeded the higher parental average in the following characters: total yield, number of seeds per plant, number of pods per plant, plant weight, height, total stem and branch length, number of nodes per plant, and number of days between planting and flowering. All these measures were good criteria of added vigor exhibited by hybrids as compared with parents.

Internode number and length have been used by many workers as measures of growth rate in plants. Anderson and

Schregardus (1), in their study of growth, noted that plants may differ from each other, not only in number of internodes and their absolute dimensions, but also in the relative size of successive internodes and in the pattern of change of relative size.

When selection is desired for a certain character, it is often desirable to determine, if possible, what other characters of the plant are associated with it. Sometimes certain characters are inherited together. By selecting for one, it may then be possible to secure the others. This association of characters frequently presents difficulties. In alfalfa, Tysdal and Westober (19) noted that selection for increased forage production usually gave at the same time a coarser and more sparsely leaved plant of poorer quality.

Wexelson (22), in his studies of inbreeding and heterosis in red clover, used plant height, weight, stem number, and internode length to measure growth. He found that no effect of inbreeding on seed weight at the time of germination could be demonstrated. However, he noted that inbreeding effects were visible at an early stage of seedling growth, inbred seedlings being in general weaker than crossbreds. This effect might appear immediately after germination or at a somewhat later stage. He found that differences in vigor between inbred and crossbred families

were retained in vegetative propagation. In his correlation studies he presented data which showed that depression in weight was significantly correlated with depression in height and that number of stems and the depression in the latter were correlated with depression in internode length. He concluded that depression of plant weight by inbreeding was caused by a reduced plant height due to shorter internodes.

In 1914 Freeman (4) studied correlations between various characters in alfalfa and among others found positive correlations indicating that nitrogen content was associated with percentage of leaves; and green weight with both the average number of stems and the average height. He found negative correlations indicating that a greater forage yield was associated with a smaller percentage of leaves, a greater height, a smaller number of stems, and a greater average height. He found no significant correlation between thickness of stand and percentage of leaves.

Hackborth and Ufer (7), in their study of growth characters in alfalfa found a high positive correlation between forage yield and height and also a positive correlation between height of stem, length of internode and coarseness of stem. Thus, a high-yielding plant was tall and upright but of low quality because it had long internodes, was sparsely leaved, and had thick woody stems. He also found a positive though slight correlation between forage production and seed production.

GROWTH CHARACTERISTICS OF ALFALFA

The alfalfa plant is a long-lived perennial, its average length of life being from five to seven years depending upon environmental conditions. It develops annually from a crown, new shoots, or stems, which normally flower and set seed. Hayward (8) and Stewart (18) have reported on the structure and development of the plant. Their observations would suggest five specific phases in the life cycle of alfalfa:

(1) syngamy to mature seed
(2) germination to seedling (first true leaves)
(3) seedling to flowering
(4) flower to mature seed
(5) second and each subsequent year's growth
    (a) origin of secondary stems to flowering
    (b) flowering to mature seed.

This study is concerned with the growth rate in the second and third phases.

The second phase represents the first independent growth of the young plant. In germination, the primary root emerges near the hilum and penetrates the soil rapidly, forming a slender, unbranched tap root. The seedling is epigeal and oval cotyledons are raised above the ground as the hypocotyl elongates and straightens. The first epicotyledonary

leaf blade is simple and obcordate or orbicular with a very slender petiole, while the second, third, and subsequent leaves are trifoliate.

At the beginning of phase three the seedling axis is slender with a short first internode, but the second internode may elongate considerably as the plant develops. There are buds in the axils of the cotyledons as well as in the axil of each trifoliate leaf, and these develop coincidentally with the growth of the primary axis to originate the so-called crown. Usually, there are three and sometimes four branches in addition to the primary axis which form this characteristic crown. When four develop the fourth arises from the axillary bud of the first trifoliate leaf, following the development of the other three from the cotyledonary buds and that of the unifoliate leaf. In the case of vigorous growing plants, additional branches may arise from adventitious buds which occur near the bases of the axillary branches. The first one usually develops between the branches arising from the buds in the axils of the cotyledons. In older plants several buds may develop each from any point in the periphery of the crown, as designated by Stewart (18) to include the perennial portion of the stems of the plant.

Alfalfa has an indeterminate growth habit. In this typical "open" growth system added vigor is expressed in the production of greater linear growth and numbers, rather

than in increase in actual size of primary organs such as leaves, stems, flowers, and seeds.

FIELD EXPERIMENT

The first of two studies on growth rate was made in the field in the summer of 1946.

Materials

The seed used for this experiment was obtained from open-pollinated and seventh-generation selfed strains. The open-pollinated strains are designated by their common varietal names and the selfed strains by the numbers under which they were recorded at the University of Alberta.

It should be pointed out that the selfed strains were not derived from the open-pollinated strains used, therefore a greater variety of material is represented than would have been the case if both selfed and open-pollinated strains had come from common origins.

The two groups, self- and open-pollinated strains, were used in order to obtain the widest possible variation in growth rates, it being presumed that the self-pollinated strains would grow less rapidly.

Experimental Methods

Five open-pollinated and five selfed strains were used. The seeds were first scarified in concentrated sulphuric acid for 18 minutes, then thoroughly washed with tap water and dried. They were soaked in petri dishes between damp filter paper on June 4, 1946, at 60°F. for 24 hours. Then they were planted on summerfallowed land on June 5, 1946, at the rate of three seeds per hill, each hill being regarded as an experimental unit or plot. The hills were check-rowed two feet apart each way and arranged in the usual random manner into two 10 x 10 adjacent Latin squares. This provided ten replicates for each strain in each Latin square.

Thirty-seven days after planting the hills were thinned to one plant each. Several hills, however, had failed to produce plants.

On September 9th and 10th, 1946, all plants were cut just above the crown and weighed.

In order to study radicle elongation and to correlate data thus obtained with weights of field-grown plants, seeds of the same experimental strains were planted in upright glass-faced cases in the greenhouse. These cases were so designed as to allow visual examination of the root at intervals throughout its early development. By stacking these cases in close contact side by side the glass faces,

and consequently the alfalfa roots, were maintained in a state of darkness necessary for normal growth.

Statistical reduction of data obtained in this investigation follows the methods outlined by Goulden (6).

Methods of Measuring Plant Characters

The stem heights of plants in the field were measured in centimeters from the cotyledonary node to the top of the longest stem. These measurements were taken twice, at the time of thinning (thirty-seven days after planting) and at the time of cutting. The fully opened flower clusters on the whole plant also were counted at this time. Each plant was weighed immediately after it was cut. The stems counted at this time included those arising in the axils of the cotyledons at the first node of the primary axis, and those from the node of the first trifoliate leaf. In the greenhouse the radicle was measured in millimeters from the point of emergence from the seed to the extreme tip seven days after planting.

Experimental Results

TABLE I

Mean radicle length at seven days

Open-pollinated			Selfed		
Strain	Number of seedlings	Radicle mean (mm.)	Strain	Number of seedlings	Radicle mean (mm.)
Ranger	19	57	5-31	19	33
Vega	16	52	1-48	21	39
Ferax	22	49	4-8	20	45
Argentine	19	52	3-48	19	42
Kansas	23	45	3-24	18	42
Means		51.2 ± 1.3			40.6 ± 1.6

The difference between radicle lengths in the two groups is highly significant.

The variability within strains grown in replication has been computed by the total variance, variance between strains, and variance within strains. From the latter are calculated standard errors of the means.

The weighted means of the measured characters for open-pollinated and selfed strains are tested for significant difference by comparing the difference between their means with the standard error of the difference and applying the "T" test. There are significant differences between the fol-

TABLE II

Means of character measurements of five open-pollinated and five selfed strains taken in the field during the summer of 1946

Strain	Number of plants	Mean number of Stems	Nodes	Flower clusters	Height 37 dy. (cm.)	Height 109 dy. (cm.)	Green wt. (gm.)
Open-pollinated							
Ranger	15	4.7	15.9	6.3	6.00	52.1	61.8
Vega	17	5.8	15.3	16.8	6.28	55.6	87.9
Ferax	14	5.2	14.8	5.6	5.37	49.5	61.2
Argentine	13	4.5	18.0	2.6	5.08	59.2	73.7
Kansas	17	5.1	15.5	4.2	5.79	55.3	69.5
Means		5.0	15.8* ±0.5	7.4** ±0.9	5.74 ±0.83	54.3** ±1.1	71.3** ±4.1
Selfed							
5-31	14	5.4	12.9	1.2	3.30	34.7	27.7
1-48	15	4.5	14.1	2.9	3.80	35.2	30.9
4-8	15	4.5	13.5	3.6	4.22	39.7	42.1
3-48	14	5.9	14.0	0.4	3.94	38.2	42.4
3-24	15	5.1	14.3	1.7	4.45	38.3	27.3
Means		5.1	13.8 ±0.3	2.0 ±0.5	3.87 ±0.66	39.4 ±0.7	32.1 ±2.5

* Mean significantly higher to the 5% point

** Means significantly higher beyond the 1% point than the corresponding means for the self-pollinated strains.

lowing means: stem height at one hundred and nine days, number of nodes, number of flower clusters, and green weights, in all cases in favor of the open-pollinated strains. There is no significant difference between the means for number of stems.

Table 12

Means of observed measurements of five open-pollinated and five mated chestnut trees in the field during the summer of 1946

Source	No.	Mean number of		Weight	Diameter	Mean
		flowers		37 dy.	108 dy.	%
Strain	Clumps	Spikes	Nuts	(gm.)	(gm.)	
Open-pollinated						
Hooper	15	4.7	12.4	7.2	4.20	58.3
Vega	17	6.2	13.4	10.0	6.20	62.0
Dana	14	6.2	14.0	9.6	5.20	54.2
Arrowline	10	4.6	10.0	6.0	3.08	51.3
Tanbee	17	6.1	10.4	8.2	4.76	58.0
Means		5.6	12.0	7.44	4.68	62.9
		±2.4	±7.0	±3.80	±1.1	±14.2
Mated						
S-21	14	4.4	11.4	5.2	3.20	58.7
I-49	15	6.8	14.2	9.0	5.80	64.4
I-4	16	6.2	13.8	9.8	6.20	63.2
S-42	14	6.1	14.2	9.2	5.84	63.4
S-24	16	4.2	12.2	7.2	4.24	58.9
Means		5.1	12.4	8.0	5.46	61.7
		±1.6	±3.2	±3.68	±2.7	±8.6

* Mean significantly higher at the 5% point

** Mean significantly higher beyond the 1% point than the corresponding means for the open-pollinated strains.

There is no significant difference between the open-pollinated and selfed groups in height of stem at thirty-seven days (P = 0.09), but the heights of stems for the open-pollinated groups are consistently higher than those for the selfed group and, for this reason, it is considered that the difference is very likely real. It is probable that this difference could have been calculated as statistically significant if the measurement had been made at a later date.

TABLE III

Coefficients of correlation between character measurements; (1) radicle length at 7 days, (2) height at 37 days, (3) height at 109 days, (4) number of nodes, (5) weight

$r_{5\ 4} = +0.738^{*}$

$r_{3\ 4} = +0.875^{**}$

$r_{5\ 3} = +0.892^{**}$

$r_{5\ 1} = +0.793^{**}$

$r_{3\ 2} = +0.892^{**}$

* Significant beyond the 5% point
** Significant beyond the 1% point

Both height at 109 days and radicle length at seven days show a highly significant positive correlation with weight. The stem height measured at 37 days shows a highly significant positive correlation with height measured at 109 days. The stem height at 109 days and node number

are positively correlated with a high degree of significance.

Discussion

As a result of measurements taken in the greenhouse it is shown that, in the same length of time, the open-pollinated strains produced longer radicles than selfed strains. The measurements of characters in the field indicate that the open-pollinated strains exceeded the selfed strains in stem length, number of nodes, time of blooming, and final green weights.

The correlations calculated indicate that the measurements of radicle length at seven days, stem height at 37 days, stem height at 109 days, yield approximately the same relative information as final weighing on the variability in plant vigor of growth for the strains used.

From the data obtained and observations made in this experiment it seems that radicle length, stem height, number of nodes, number of open flower clusters, and final weight, may be good criteria by which to differentiate growth rate in open-pollinated and selfed strains of alfalfa.

GREENHOUSE EXPERIMENT

Since only one year's results were obtained from the field experiment, it was thought that, though greenhouse experimental results are not always fairly comparable with those obtained under field conditions, some additional valuable data on growth measurements might be obtained from a greenhouse experiment. It was also desirable to determine whether the results obtained in the field could be substantiated by similar measurements taken under greenhouse conditions.

Materials

Seeds from three of the five open-pollinated strains used in the field were employed here. These were Ranger, Ferax, and Kansas. Owing to a shortage of seed, different selfed strains from those used in the field had to be utilized. They were similar to those used in the field experiment in that they were produced under the same conditions, but were of different stock material. The seeds were from seventh generation selfed plants.

Methods

The studies on the methods of measuring growth in alfalfa were made in the greenhouse with a temperature range of 65° to 75°F. during the winter of 1946-47. The soil used in the pot cultures was a sterilized mixture consisting of one-fourth sand and three-fourths Edmonton black loam. No supplementary nutrients were added to the soil. The natural daily light period was increased to sixteen hours by means of artificial lighting.

The testa of seeds used was nicked with a razor blade and placed between damp filter paper in petri dishes at a temperature range of 65° to 75°F. for seventy-two hours. The germinated seeds were inoculated with root nodule bacteria (Rhizobium meliloti) isolated from nodules of alfalfa. The most vigorous germinated seeds were transplanted, three in a pot, on November 5th, to the previously prepared soil in ten-inch pots.

The pots were arranged so that they constituted a complete randomized block experimental design with four replications of six strains. Each strain "plot" consisted of six pots, thus making a total of 36 pots in each replicate.

Twenty-five days after planting, the seedlings in the pots were thinned to two plants per pot. The seedlings

taken from the pots were weighed and measured for stem length. The fully opened trifoliate leaves were also counted for each plant. The stems were measured from the cotyledonary node to the tips of the uppermost leaves when these were extended by gentle upward stroking.

On February 14th--that is, one hundred and one days after transplanting--plant heights were measured from the soil surface in the pot to the tip of the stem. Stems ten centimeters and over in height were the only ones measured. The nodes were counted on all measured stems.

All plants were cut just above the cotyledonary node and weighed individually in grams on February 17th, 104 days after transplanting.

Experimental Results

Before proceeding with the above-described experiment, a comparison was made of the weights of open-pollinated and the selfed seed in order to determine whether differences in subsequent growth rates might be related to differences in original seed weights.

TABLE IV

Weight of 100 seeds in grams used in the greenhouse growth measurement studies

Open-pollinated		Selfed	
Strain	Weight in grams per 100 seeds	Strain	Weight in grams per 100 seeds
Kansas	0.18	21-7	0.24
Ferax	0.26	15-34	0.23
Ranger	0.18	3-32	0.22
Mean	0.21 ± 0.03	Mean	0.23 ± 0.01

There is no significant difference between the mean weight per 100 seeds of the open-pollinated and the selfed groups, and therefore any difference in later growth cannot be attributed to difference in initial seed weight. There is a greater variation in seed weight among the open-pollinated strains, as might be expected from the variability among different varieties.

TABLE V

Measurements on seedlings removed from pots
25 days after transplanting

Strain	Number	Weight per seedling (gm.)	Stem length (cm.)	Number of leaves
Open-pollinated				
Ferax	22	0.6	4.7	1.7
Kansas	21	0.6	5.4	1.7
Ranger	21	0.5	5.0	1.9
Mean			5.0±1.6	
Selfed				
3-32	19	0.5	4.2	1.8
15-34	21	0.7	4.3	1.8
21-7	24	0.6	4.8	1.4
Mean			4.5±1.4	

In this experiment the means and their standard errors were calculated for the measured characters for open-pollinated and selfed groups and the means tested for significance, in the same manner as described for Table II.

There is no significant difference between open-pollinated and selfed groups in any of the characters measured at this stage. However, the data suggest slightly greater stem length for the open-pollinated group.

TABLE VI

Means of character measurements of six strains of alfalfa taken in the greenhouse on plants 104 days old

Strain	Weight (gm.)	Length of longest stem (cm.)	Total length of stem per plant (cm.)	Av. length of stem per plant (cm.)	No. of stems over 10 cm.	No.of nodes per longest stem	Av.no. of nodes per stem
Open-pollinated							
Ferax	2.9#	30.2	74.4	22.4	3.2	13.0	10.7
Kansas	3.3	34.4	97.1	28.3	3.5	12.9	10.8
Ranger	3.3	34.1	99.7	24.7	4.0	12.9	10.1
Means	3.2 ±0.7	32.9** ±1.0	90.4** ±3.2	25.5* ±0.7	3.6** ±0.1	12.9	10.5 ±0.4
Self-pollinated							
3-32	2.5	24.8	47.9	20.7	2.5	12.3	10.9
15-34	1.3	24.5	40.9	23.7	1.8	12.9	11.9
21-7	2.4	21.9	27.5	20.6	1.4	13.5	12.5
Means	2.1 ±0.8	23.6 ±0.7	38.7 ±2.4	21.6 ±0.6	1.8 ±0.1	12.9	11.6 ±0.7

Each value is the mean of four replicates.

* Mean significantly higher to the 5% point
** Means significantly higher beyond the 1% point than the corresponding means for the self-pollinated strains.

There is a highly significant difference between the following means: length of longest stem, total length of stem per plant, and number of stems ten centimeters and over, in all cases in favor of the open-pollinated group. There is a significant difference in average length of stems, the open-pollinated group producing the longest stems. The differences for weight and number of nodes per measured stem between the two groups are not significant.

TABLE VII

Coefficients of correlations of measured characters, (1) weight, (2) mean length of stem, (3) mean number of stems over 10 centimeters, (4) total stem length, (5) length of longest stem, taken on greenhouse plants 104 days old

$$r_{1\,2} = .973^{**}$$

$$r_{1\,3} = .970^{**}$$

$$r_{1\,4} = .982^{**}$$

$$r_{1\,5} = .980^{**}$$

** significant beyond the 1% point.

The correlation coefficients given above indicate highly significant positive correlations between weight and the following variables in Table VI: lenght of stem, number of stems over ten centimeters, total stem length per plant, and length of longest stem.

Most of the variability in plant weight could obviously be accounted for by its association with any one of the afore-named characters. These correlations indicate that by measuring any of these characters the same relative information could be obtained on growth rates as is obtained from plant weight.

Discussion

Examination of Table V suggests that, except for height of stem, there seems to be no difference between the open-pollinated and selfed strains at this early stage of growth, viz. 25 days. The data indicate that the open-pollinated strains may have the longer stems at this stage.

The final weights of open-pollinated and selfed strains (Table VI) are not shown to be statistically different, but the differences are so consistently in favor of the open-pollinated strains that it seems fairly safe to postulate that the difference is real.

There is a highly significant difference in stem lengths between the two groups (Table VI) but there is no difference in the average number of nodes per stem. This same relationship was observed on number of nodes per longest stem. It would seem that at this stage of growth increase in stem length may be due more to an increase in

internode length than to an increase in node number.

The data from this experiment indicate that growth measurements such as weight, total stem length, length of longest stem, average length of stem, and average number of stems over ten centimeters in length, are all good criteria of growth in alfalfa under greenhouse conditions.

Correlations between weight and the above-mentioned measured characters are highly significant. It seems that any of these measures will demonstrate the same relative information on growth rates. Under greenhouse conditions stem length appears to be the best indicator of growth-rate differences which exists between open-pollinated and selfed strains of alfalfa.

GENERAL DISCUSSION

The weight of forage produced has been used as the principal criterion of growth and vigor in alfalfa; consequently, final plant weight was used as one of the measures of growth in these experiments. In experiments reported herein other measurements were made and correlated with weight to test their comparative value as growth measures. Green weights were used as it was desired to get a standard of size increase. There was greater variability in green weights in plants

grown under field conditions than in those grown under greenhouse conditions. Plant weight was positively correlated with length of stem, number of stems, number of nodes, and length of radicle. These correlations indicate that criteria other than weight measures could be used for studies of growth rates in alfalfa.

In these studies the earliest difference noted between the open and selfed strains was that of the linear growth of the radicle of seven-day-old seedlings. This character was easily measured and seems to be a reliable index of existing differences in potential growth rates. There was no significant difference in the weights of seeds used, but it may be possible that there would have been a difference in seed weights if all lots had been produced under similar conditions.

The plant height, as measured by stem length, was a good criterion of growth in alfalfa. Measurements of this character demonstrated that existing differences in growth rates of alfalfa plants could be established at an early stage. The differences in growth rate between inbred and open-pollinated strains were shown at the time of final weighing in both field and greenhouse experiments. The total length of stems produced per plant, as measured in the greenhouse, indicated that open-pollinated strains produced more aerial growth than selfed strains. Plant height is

positively correlated with weight, which indicates that heavier plants produced longer stems.

In alfalfa, leaves, lateral stems, buds, and flowers originate at the nodes. Therefore, it would be expected that as the number of nodes per plant increased so would the production of leaves, lateral stems, pods, and seeds.

In the field experiment the nodes, as counted on the longest stem, were more numerous for the open-pollinated strains, but this was not the case in the greenhouse. This discrepancy may be due to differences in stage of growth.

As a result of measurements taken in the field experiment and observations made in the greenhouse, it seems that the number of basal stems that plants were able to produce did not differ appreciably. However, when total length of stems produced was measured, it seemed that the more vigorous plants produced their basal stems earlier, and these grew longer in the same length of time. The total length of stems measured under greenhouse conditions indicated that open-pollinated plants had the greater total stem length.

The data on the earliness of floweringare meagre. Observations of this characteristic in the field indicated that open-pollinated strains were earlier blooming than the selfed strains, and it seems likely that they would consequently be earlier maturing.

The data from these experiments indicate that the

best criteria of growth rates for the alfalfa strains used were: green weights, plant height, total stem length, and radicle length.

SUMMARY

1. Alfalfa growth rate was measured in terms of radicle elongation, stem number, stem length, and node number, at specified time intervals, after placing the seeds under germination conditions, and these characters were correlated with weight of aerial growth.

2. The difference in growth rates between open-pollinated and selfed strains was measured in the greenhouse at the seedling stage by radicle elongation.

3. Early in the development of the plants there was a differential growth rate between open-pollinated and selfed strains, as shown by measurements of stem length in both the field and greenhouse experiments.

4. When the plants of the field experiment were harvested there were differences observed in length of longest stem, number of nodes, number of open flower clusters, and weight of plants, in favor of the open-pollinated group in all cases.

5. At the time plants were harvested in the greenhouse there were significant differences in length of longest stem, total length of stem, number of stems exceeding ten centimeters in length, between open-pollinated and selfed strains. This corroborates the evidence of superiority of the open-pollinated strains in the field experiment. There was no significant difference in weights between the open-pollinated and selfed strains, but it is considered that the difference is real and in favor of the open-pollinated groups. There was no inter-group difference in the number of nodes per measured stem.

6. Under conditions of these experiments, length of radicle, length of longest stem, total stem length, number of stems over ten centimeters, were the best criteria of growth rates for the strains of alfalfa used in this study.

7. From the high correlations between these growth characters and weight it would appear that any of these may be used as a substitute for weight of aerial parts in estimating the relative rates of growth of alfalfa plants.

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